I. Drawings:

The Examiner's objection of the drawings is duly noted. The Applicants will attend to filing formal drawings at an appropriate time later in prosecution phase of the present application.

II. Objections to the Specification:

The Examiner has objected to the specification in view of a number of minor inadvertent typographical errors. The specification has been amended to correct these errors. Since no new matter has been added, entry of the amendments to the specification is respectfully requested.

In light of the above, it is believed that the objections to the specification have been rendered moot and withdrawal thereof is respectfully requested.

III. 35 U.S.C. § 112, Second Paragraph Rejection:

Claims 1 to 5 have been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not adequately described in the specification in such a way as to enable one skilled in the art to make and/or use the invention.

With regard to this rejection the Examiner has raised a number of issues, which include: (1) that the specification teaches treatment of only two bacteria, *M. tuberculosis* and *S. typhimurium*; (2) the specification provides no data resulting from the procedures disclosed therein relating to the treatment of the above bacteria in mice (as such the Examiner contends that there is no actual vaccine evidence); and (3) that due to the lack of evidence/data relating to other diseases (listeria, HIV, cancer, etc.) the specification is non-enabling for vaccines against these diseases as well.

With regard to points 1 to 3, it is pointed out that in view of the information contained in the application as filed and the level of skill which can be attributed to one of ordinary skill in the art, claims 1 to 3 and 5 are enabled.

This is because the application as filed discloses how to produce a vaccine against: (1) TB and other intracellular pathogens (Example 1), (2) TB (Example 2), and (3) salmonella. Accordingly, upon reading and understanding the application as filed, one of ordinary skill in the art would readily appreciate how to utilize the disclosed method to produce vaccines against the a wide variety of diseases as recited in claims 1 to 3 and 5.

As is well known, the Applicants are permitted to claim their invention as broadly as is supported by the specification and figures as filed. Accordingly, absent any specific evidence to the contrary, the Applicants believe that claims 1 to 3 and 5 are fully enabled by the application as originally filed. Accordingly, withdrawal of the 35 U.S.C. § 112, first paragraph rejections of claims 1-3 is respectfully requested.

Furthermore, it is pointed out that it is well known to those of ordinary skill in the art that certain diseases, including cancer, are caused by intracellular infectious pathogens (*e.g.*, cervical cancer has been shown to be linked *Human papillomavirus* (HPV)).

IV. 35 U.S.C. § 112, Second Paragraph Rejections:

Claims 1 to 3 and 5 have been rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Specifically, the Examiner contends that the listing of the cell lines in part (ii) of claims 1 to 3 is indefinite due to the use of etc. and "J774A". Claims 1 to 3 have been amended to recite "J774" rather than "J774A" and to remove the word etc. Accordingly, the above rejection is believed to have been rendered moot in light of the amendments made to claims 1 to 3, and withdrawal thereof is respectfully requested.

Claim 4 has been rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Specifically, the Examiner contends that claim 4 is indefinite due to the word "substantially" and the reference to the Examples. This rejection has been rendered

moot in view of the cancellation of claim 4. Accordingly, withdrawal thereof is respectfully requested.

Claims 1 and 5 have been rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Specifically, the Examiner contends that in claim 1, the phrase "with known drugs" is indefinite since one of ordinary skill in the art would not be able to determine from the claim the criteria for choosing such drugs. With regard to claim 5, the Examiner contends that the phrase "by the available drugs against the pathogens" is indefinite because, again, one of ordinary skill in the art would not be able to determine from the claim the criteria for choosing which drugs to use.

With regard to claim 1, claim 1 has been amended to remove the word "known". As such, it is now believed that claim 1 is in compliance with 35 U.S.C. § 112, second paragraph and withdrawal of this rejection is respectfully requested.

With regard to claim 5, it is pointed out that one of ordinary skill in the art would recognize in light of the pathogen to which a vaccine is being produced which drugs are available against such a pathogen. As such, it is now believed that claim 5 is in compliance with 35 U.S.C. § 112, second paragraph and withdrawal of this rejection is respectfully requested.

Claims 1, 4 and 5 have been rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Specifically, the Examiner states that it is unclear as to why the Applicants have categorized cancer and tumors as intracellular pathogens.

As noted above, it is known to those of ordinary skill in the art that certain cancers (e.g., cervical cancer) have been linked to various intracellular pathogens. Accordingly, it is believed that claims 1 and 5 are definite in view of the disclosure contained in the application as filed and in light of the level of skill attributable to one of ordinary skill in the art. Accordingly, the rejection of claims 1 and 5 under 35 U.S. C. § 112, second paragraph, is believed to be unwarranted and should be withdrawn.

V. Conclusion:

In view of the above, withdrawal of the above-mentioned objections and rejections and allowance of claims 1 to 3 and 5 is respectfully requested.

Should the Examiner believe that a telephone interview would be helpful to expedite favorable prosecution, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

In the event any fees are due in connection with the filing of this document, the Commissioner is authorized to charge those fees to our Deposit Account No. 18-0988, Attorney Docket No. **KUMAP0105US**.

Respectfully submitted,
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APPENDIX

Below is a detailed listing of the changes made to the specification and claims. Please note, underlining denotes additions and [~~bracketed strikeout~~] denotes deletions.

In The Specification:

The following passages of the specification have been amended as shown below.

Page 1, lines 12 to 19:

The utility of the present invention is to develop a vaccine against the intracellular pathogens, which are causative agents of tuberculosis, brucellosis, leishmaniasis, [~~leisteriosis~~] listeriosis, leprosy, malaria, typhoid, trypanosomiasis and streptococcus and HIV-infection. The pathogen *Mycobacterium tuberculosis* (*M. tuberculosis*) the subject matter of this invention is a causative agent of tuberculosis. In this invention *M. tuberculosis* was allowed to grow in the allogeneic and syngeneic macrophages and macrophage cell lines. The macrophages-*M. tuberculosis* complex was then irradiated to kill the macrophages as well as the mycobacterium.

Page 2, line 23 to page 3, line 3:

A new question has arisen regarding the safety of BCG in HIV-infected individuals. A small number of cases of disseminated BCG-osis have been reported among children who received BCG vaccine and were subsequently found to be HIV seropositive (Von Reyn, et. al. *Lancet* 1987: ii:669-672; Braun, et. al., *Pediatr. Infect. Dis. J.* 1992:11:220-227; Weltman, et. al., *AIDS* 7:1993:149). WHO currently recommends discontinuing the use of BCG vaccine in children showing overt [~~over~~] signs of immunodeficiency (World Health Organization, 1992, *Expanded Program for Immunization, Program Report*,World Health Organization, Geneva; *Weekly Epidemiol. Rec.* 62:1987:53).

Page 6, lines 8 to 18:

The main rationale behind this process was to develop a vaccine against tuberculosis and other intracellular diseases, MHC-matched (syngeneic) and mismatched (allogeneic) macrophages harboring *M. tuberculosis* on irradiation undergo apoptosis; dendritic cells engulf these macrophages and present the antigen (Mycobacterium-proteins and allo-

macrophage peptides) on their surface and induce naïve T-cells to differentiate into effecter $CD4^+$ Th1 cells. These dendritic cells also activate $CD8^+$ T cells for cell-mediated immunity. Allo-macrophages in the system generate an allo-reaction and as a result a large amount of cytokines like IL-2, IL-12, IFN-γ, etc., are produced which promote the Th1 response and cell mediated immune response. It is known that Th1-type of response provides protection against tuberculosis. Hence the main utility of the process was to produce a potent and specific vaccine against *M. tuberculosis*.

Page 6, lines 21 to 24:

The main object of the present invention thus is to develop a vaccine against tuberculosis and other intracellular diseases like leprosy, leishmaniasis, typhoid, trypanosomiasis, malaria, brucellosis, [~~leisteriosis~~] listeriosis, AIDS, streptococcal infection and cancer.

Page 7, lines 5 to 10:

Another object is to develop a vaccine that acts against both syngeneic macrophages entrapped pathogens (viz. M. tuberculosis, M. leprae, leishmania, salmonella, trypanosoma, malaria, brucella, [~~leisteria~~] listeria, HIV, [~~streptococcas~~] streptococcus) (e.g. SMTV, S=syngeneic, M=macrophage, T=tuberculosis, V=vaccine) and allogeneic-macrophages entrapped pathogen vaccine (e.g., AMTV, A=allo, M=macrophage, T=tuberculosis, V=vaccine), to generate protective immune response.

Page 8, lines 1 to 5:

The vaccine was used after irradiation and the irradiated cells are known to undergo apoptosis. The cells undergoing apoptosis were engulfed by the dendritic cells. Dendritic cells activated naïve T cells to differentiate into Th1 cells and cytotoxic cells. These [~~The~~] cells are known to be cardinal in imparting protective immunity against intracellular infections and cancer.

Page 13, line 12 to page 14, line 5:

Accordingly, the present invention provides a vaccine against tuberculosis and other intracellular pathogens selected from the group consisting of *Mycobacterium leprae, leishmania, salmonella, trypanosoma, plasmodium, brucella, [~~leisteria~~] listeria, HIV, [~~streptococcas~~] streptococcus* and *cancer*. The invention also provides a method for the development of the said vaccine, comprising the steps of:

(i) culturing pathogens selected from the group comprising *Mycobacterium tuberculosis*, *Mycobacterium leprae, leishmania, salmonella, trypanosoma, plasmodium, brucella, [~~leisteria~~] listeria, HIV,* and *[~~streptococcas~~] streptococcus*;
(ii) culturing syngeneic (same strain), allogeneic (different strain) and xenogeneic (different species like sheep and goat) macrophages and macrophage cell lines selected from the group consisting of J774[~~A~~], P388D1, RAW, BMC-2, THP-1, etc.;
(iii) infecting macrophages and cell lines with a pathogen;
(iv) treating the infected cells with known drugs followed by gamma irradiation to obtain the vaccine;
(v) immunizing disease resistant and susceptible strains of animals with the vaccine obtained above;
(vi) infecting the animals with live pathogen and monitoring their mortality and viable counts of infectious agent in lungs, spleen and liver; and
(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity.

Page 14, lines 6 to 26:

The invention further provides a process for the preparation of a vaccine against tuberculosis, wherein the said process comprising the steps of:

(i) culturing of *Mycobacterium tuberculosis* H37Rv;
(ii) culturing of syngeneic and allogeneic macrophages and macrophage cell lines selected from the group consisting of J774[~~A~~], P388D1, RAW, BMC-2, THP-1, etc.;
(iii) infecting macrophages and cell lines (J774, P388D1, RAW, BMC-2, THP-1) with *M. tuberculosis*;
(iv) treating the infected cells with isoniazid and gamma irradiation to obtain the vaccine;
(v) immunizing tuberculosis resistant and susceptible strains of mice with allogeneic macrophage tuberculosis vaccine (AMTV) and syngeneic macrophage tuberculosis vaccine (SMTV) obtained above;

(vi) infecting the mice with live *M. tuberculosis* and monitoring their mortality and viable counts of bacteria in lungs, spleen and liver;
(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity; and
(viii) inoculating the vaccine in the mouse footpad and examining the delayed type hypersensitivity reaction by measuring the swelling in the footpad for protective immunity.

Page 14, line 27 to page 15, line 18:

The invention also provides a process for the preparation of a vaccine against salmonella, wherein the said process comprising the steps of:

(i) culturing of *Salmonella typhimurium*;
(ii) culturing of syngeneic and allogeneic macrophages and macrophage cell lines selected from the group consisting of J774[A], P388D1, RAW, BMC-2, THP-1, etc.;
(iii) infecting macrophages and cell lines (J774, P388D1, RAW, BMC-2, THP-1) with *S. typhimurium*;
(iv) treating the infected cells with mitomycin C and gamma irradiation to the obtain vaccine;
(v) immunizing tuberculosis resistant and susceptible strains of mice with the vaccine obtained above;
(vi) infecting the mice with live *S. typhimurium* and monitoring their mortality and viable counts of bacteria in lungs, spleen and liver;
(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity; and
(viii) inoculating the vaccine in the mouse footpad and examining the delayed type hypersensitivity reaction by measuring the swelling in the footpad for protective immunity.

Page 15, line 27 to page 16, line 3:

The intracellular pathogens viz. Mycobacterium tuberculosis, Mycobacterium leprae, leishmania, salmonella, trypanosoma, plasmodium, brucella, [~~leisteria~~] listeria, HIV, [~~streptococcas~~] streptococcus were cultured in the macrophages of syngeneic and allogeneic mice, macrophages cell lines J774, P338D1, RAW, BMC-2, THP-1 (ATCC, Rockville). The infected cells were treated with isoniazid (20 µg/ml) for 48h at 37 °C/5% CO_2 and irradiated at 0.05 kGy.

In The Claims:

The changes to claims 1-3 and 5 are shown below.

1. (Amended) A process for the preparation of a vaccine against tuberculosis and other intracellular pathogens selected from the group consisting of *Mycobacterium leprae, leishmania, salmonella, trypanosoma, plasmodium, brucella, [~~leisteria~~] listeria, HIV, [~~streptococcas~~] streptococcus* and *cancer*, wherein the process comprises the steps of:

(i) culturing pathogens selected from the group comprising of *Mycobacterium tuberculosis, Mycobacterium leprae, leishmania, salmonella, trypanosoma, plasmodium, brucella, [~~leisteria~~] listeria, HIV* and *[~~streptococcas~~] streptococcus;*

(ii) culturing syngeneic (same strain), allogeneic (different strain) and xenogeneic (different species like sheep and goat) macrophages and macrophage cell lines selected from the group consisting of J774[~~A~~], P388D1, RAW, BMC-2 and THP-1[~~, etc.~~];

(iii) infecting macrophages and cell lines with a pathogen;

(iv) treating the infected cells with [~~known~~] drugs followed by gamma irradiation to obtain the vaccine;

(v) immunizing disease resistant and susceptible strains of animals with the vaccine obtained above;

(vi) infecting the animals with live pathogen and monitoring their mortality and viable counts of infectious agent in lungs, spleen and liver; and

(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity.

2. (Amended) A process for the preparation of a vaccine against tuberculosis, the process comprising the steps of:

(i) culturing *Mycobacterium tuberculosis* H37Rv;

(ii) culturing syngeneic and allogeneic macrophages and macrophage cell lines selected from the group consisting of J774[~~A~~], P388D1, RAW, BMC-2 and THP-1[~~, etc.~~];

(iii) infecting macrophages and cell lines (J774, P388D1, RAW, BMC-2, THP-1) with *M. tuberculosis*;

(iv) treating the infected cells with isoniazid and gamma irradiation to obtain the vaccine;

(v) immunizing tuberculosis resistant and susceptible strains of mice with allogeneic macrophage tuberculosis vaccine (AMTV) and syngeneic macrophage tuberculosis vaccine (SMTV) obtained above;

(vi) infecting the mice with live *M. tuberculosis* and monitoring their mortality and viable counts of bacteria in lungs, spleen and liver;

(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity; and

(viii) inoculating the vaccine in the mouse footpad and examining the delayed type hypersensitivity reaction by measuring the swelling in the footpad for protective immunity.

3. (Amended) A process for the preparation of a vaccine against salmonella, the process comprising the steps of:

(i) culturing *Salmonella typhimurium*;

(ii) culturing syngeneic and allogeneic macrophages and macrophage cell lines selected from the group consisting of J774[~~A~~], P388D1, RAW, BMC-2 and THP-1[~~, etc.~~];

(iii) infecting macrophages and cell lines (J774, P388D1, RAW, BMC-2, THP-1) with *S. typhimurium*;

(iv) treating the infected cells with mitomycin C and gamma irradiation to the obtain vaccine;

(v) immunizing tuberculosis resistant and susceptible strains of mice with the vaccine obtained above;

(vi) infecting the mice with live *S. typhimurium* and monitoring their mortality and viable counts of bacteria in lungs, spleen and liver;

(vii) monitoring the vaccinated animals for proliferation and generation of $CD4^+$ Th1 and Th2 cells and $CD8^+$ cytotoxic T cells indicating the generation of cell mediated immunity; and

(viii) inoculating the vaccine in the mouse footpad and examining the delayed type hypersensitivity reaction by measuring the swelling in the footpad for protective immunity.

5. (Amended) A vaccine as prepared by the process of claim 1, wherein by entrapment of *M. tuberculosis, Salmonella* and other intracellular pathogens in the allogeneic and syngeneic macrophages, the preparations being [~~were~~] treated by the available drugs against the pathogens and the vaccine is further gamma irradiated before using for the protection against the infectious diseases and cancer.